(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 424 064 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: **90311253.0**

(22) Date of filing: **15.10.90**

(51) Int. Cl.5: **C07D 209/52**, C07C 233/52, C12N 1/00, C12N 9/86, C12P 41/00

The applicant has filed a statement in accordance with Rule 28 (4) EPC (issue of a sample only to an expert). Accession number(s) of the deposit(s): NCIMB 40249, 40213.

(30) Priority: **16.10.89 GB 8923278**
27.10.89 GB 8924209
17.01.90 GB 9000995

(43) Date of publication of application:
24.04.91 Bulletin 91/17

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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(54) Chiral compounds.

(57) Lactamase of 1-amino-3-carboxylic acid cyclic compounds are provided in enantiomeric form, together with an enantiomer of the corresponding ring-opened amino-acid or ester, by reaction of the racemic lactam with a novel lactamase. The products are useful in the synthesis of chiral carbocyclic nucleotides.

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CHIRAL COMPOUNDS

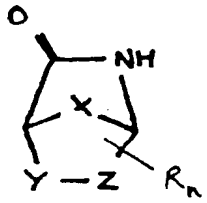
Field of the Invention

This invention relates to chiral compounds having utility as intermediates in the synthesis of anti-viral agents, and to a process for their preparation.

Background of the Invention

Various 9-substituted purines are known as anti-viral and anti-neoplastic agents. One such compound, known as AZT, has been used for the treatment of AIDS. More recent examples are disclosed in EP-A-4268672, US-A-4742064, and also in GB-A-2217320 which, by way of specific illustration, describes a compound known as Carbovir (carbocyclic 2′,3′-didehydro-2′,3′-dideoxyguanosine). Not surprisingly, the enantiomers of such chiral compounds have different activities.

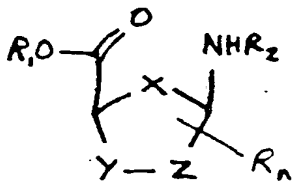
Carbovir and known analogues are prepared from the known γ-lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one, i.e. the compound of formula



I

in which X is $-CH_2-$, -Y-Z- is -CH = CH-, and R_n is absent. The prior art indicates that the final product, or any intermediate or starting material, may be resolved by known methods, and that a racemic mixture of the product may be enzymatically converted to chirally pure compounds. The γ-lactam can be prepared by reacting cyclopentadiene with tosyl cyanide.

R.D. Allan et al , Eur. J. Pharmacol. 122 (1986) 339-348, disclose a series of GABA analogues, resolved and unresolved, including compounds of formula II



II

in which -X- is $-CH_2-$, -Y-Z- is -CH = CH-, and R_1 and R_2 are H. In particular, (+)-1R,4R-4-aminocyclopent-2-ene-1-carboxylic acid is disclosed, although its activity is much lower than that of the structurally-isomeric 1-ene.

Summary of the Invention

The present invention is based on the surprising discovery of lactamases that will react with a γ-lactam of formula I to give a single enantiomer of the lactam and the corresponding ring-opened compound of formula II in an enantiomeric form. The enantiomers are novel compounds, and are excellent synthons for a desired enantiomer of Carbovir or a pharmacologically-active analogue.

In compounds of the invention, X is $-CH_2-$, $-(CH_2)_2-$, -Q-, $-CH_2$-Q- or -Q-CH_2- and Q is a heteroatom (including NH); either Y or Z are independently selected from $-CH_2$-and a heteroatom (including NH), or -Y-Z- is -CH = CH-, -CH = N- or -N = CH-; and R_n is absent or represents one or more independently-selected

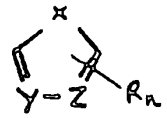
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substituents at any available position(s) on the X,Y,Z-containing ring. R_1 is H or alkyl; R_2 is H or a blocking group.

While (+)-4-aminocyclopent-2-ene-1-carboxylic acid is known, the present invention provides a new utility, i.e. its conversion to another compound of formula II, e.g. as a synthon.

Description of the Invention

Unsaturated lactams of formula I (in which -Y-Z- means -CH=CH-, -CH=N- or -N=CH-) can be prepared, by generally known means, by a Diels-Alder reaction which comprises reacting a corresponding compound of formula III



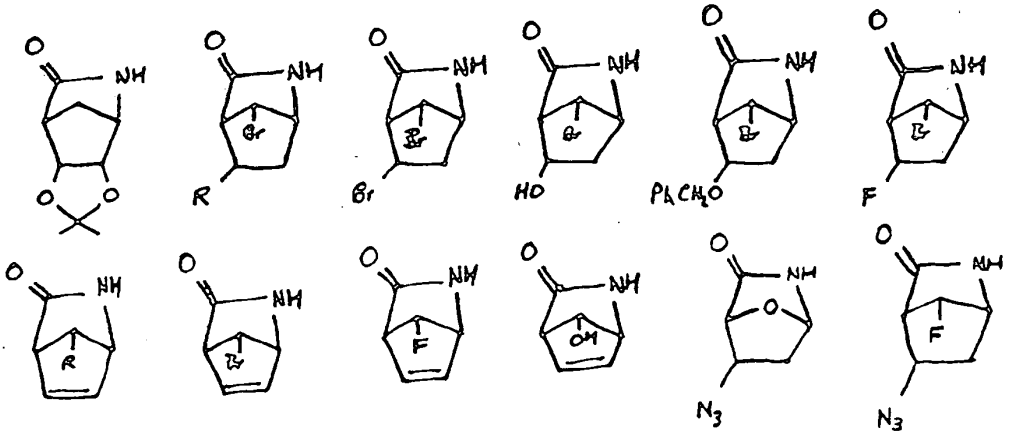
III

with tosyl cyanide or chlorosulphonyl isocyanate. Depending on the nature of X, the compound of formula III may variously be, for example, a cyclopentadiene, 1,3-cyclohexadiene, furan, pyrrole or 1,2-dihydropyridine.

Unsaturated lactams of formula I may be reduced to give saturated lactams of formula I, if necessary with concomitant protection of susceptible groups, by known means. The unsaturated lactams may also be utilised in the preparation of compounds of the invention bearing one or more substituents R.

R_n may be any substituent or substituents which do not interfere with the lactamase reaction; examples of R (if present) are methyl, ethyl, n-butyl, OH, Cl, Br, F, CF_3 and azido. For example, F or another halogen may be a substituent of X, e.g. as -CHHal-. The total number of carbon atoms in the group or groups R will not usually exceed 8. n may be, for example, 1 or 2, but R_n is preferably absent.

The compound of formula III is preferably a 1,3-cyclohexadiene or, most preferably, a (symmetrical) 5-membered compound. X is preferably $-CH_2-$. Specific examples of compounds of formula I are the following:



Others are given in PCT/GB90/00574.

It is surprising that material of biological origin will react selectively with a γ-lactam of formula I, to give the desired products in good yield. The material is described herein, for convenience, as a lactamase.

Suitable activities are those present in certain novel wild-type isolates of the genera Pseudomonas, Alcaligenes, Arthrobacter, Brevibacterium, Nocardia, Rhodococcus and Corynebacterium, whilst not being limited to isolates of these genera. Selection for these activities may be conducted in the presence of compounds containing one or more N-acyl substituents. If necessary, elevated levels of activity may be produced by growth of cells in the presence of such compounds. Particular examples of suitable activity are those produced maximally active in cells of the unique strains ENZA-20 and Rhodococcus sp ENZA-1, the latter when cultivated in a suitable medium in the presence of N-acetyl-L-phenylalanine or N-acetyl-D,L-

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phenylalanine.

Rhodococcus sp ENZA-1 was isolated from soil samples by enrichment culture in mineral salts medium containing N-acetyl-L-phenylalanine as the sole source of carbon and energy. The isolate has been deposited at the NCIMB in Aberdeen, on 17.10.89. The accession number is NCIMB 40213.

ENZA-20 was obtained from a sewage sample, and deposited at the NCIMB on 16.01.90. The accession number is NCIMB 40249.

Novel microorganisms have the ability to cause stereoselective formation of enantiomers of, say, 2-azabicyclo[2.2.1]hept-5-en-3-one and (-)-4-aminocyclopent-2-ene-1-carboxylic acid. The deposited microorganisms may, if desired, be subjected to conventional mutagenic/selection procedures, with a view to attaining improved effects.

The reaction of the lactam with the material having lactamase activity gives a compound of formula II which can be separated, as necessary or desired, from admixture with the unreacted lactam. If desired, the product (R_2 = H) can be reacted with an acylating agent such as acetic anhydride, to give the corresponding compound (II: R_2 = acyl). Other conventional N-blocking groups can be introduced as desired, examples being CH_2Ar, COOalk, CONHalk, SO_2alk, Si(alk)$_3$, CHO and COalk (alk meaning alkyl in its broadest sense).

If the lactamase reaction is conducted in the presence of water, R_1 is H. Alternatively, a nucleophile may be used to introduce an alkyl or other group R_1 directly. For example, the nucleophile is methanol.

(-)-4-Aminocyclopent-2-ene-1 carboxylic acid and other cis compounds of formula II are often the product of the process. If desired, racemic cis amino-acid may be converted to racemic cis/trans amino-acid which is then exterified, allowing ready separation. The, say, (+)-trans ester may be isolated.

One alternative synthesis of the amino-acids (II) is by cleavage of the racemic lactam (I) with a chiral nucleophile (e.g. an amine or alcohol). The cleavage may show some entantioselectivity, and the resultant diastereoisomers can be separated by fractional crystallisation. Acid hydrolysis (if needed) liberates the free amino-acid.

A further alternative comprises enantioselective reaction on the double bond. Potential examples are

(i) asymmetric hydroboration (as pioneered by H.C. Brown)

(ii) catalytic asymmetric dihydroxylation (B. Sharples)

(iii) binaphthyl rhodium/ruthenium-catalysed double bond displacement (Noyori; such a process is used in tonne scale menthol manufacture).

In each case, the unreacted lactam could be collected or the functional product be used to develop a route to a chiral drug.

Yet another alternative route to chiral products of the invention comprises conducting the Diels-Alder addition with a chiral Lewis acid catalyst or by using a chiral dienophile in place of tosyl cyanide. Economically, the use of a chiral catalyst is preferred. The resultant optically-enriched lactam may be subjected to the novel biotransformation process, in order to obtain a higher yield of pure (-)-lactam than may be possible from the racemate.

The optical isomers of the lactam (I) have a higher melting point than the racemate and therefore a higher lattice energy. Enantiomers may therefore be obtained by preferential crystallisation (entrainment), i.e. by seeding a supersaturated solution of racemate in a suitable solvent with pure enantiomer, such that more enantiomer is recovered than put in.

The following Examples illustrate the invention.

Example 1

Preparation of Cells

Rhodococcus sp ENZA-1 was grown in a medium containing KH_2PO_4 (7 gl^{-1}), Na_2HPO_4 (2 gl^{-1}), $MgSO_4$ (0.4 gl^{-1}), NaCl (2 gl^{-1}), $CaCl_2.6H_2O$ (0.01 gl^{-1}), $FeCl_3.7H_2O$ (0.08 gl^{-1}), $ZnSO_4.7H_2O$ (0.0001 gl^{-1}), $(NH_4)_2SO_4$ (2 gl^{-1}) yeast extract (1 gl^{-1} and glucose (10 gl^{-1}). The medium was adjusted to pH 7.0 with 5M NaOH and sterilised by autoclaving at 121 °C for 20 min. N-acetyl-L-phenyl-alanine at pH 7 was filter-sterilised and added to the cooled medium to give a final concentration of 10 gl^{-1}. The medium was distributed in 1 litre volumes within 5 litre shake flasks and 100 ml volumes within 500 ml shake flasks. A loopful from a slant of ENZA-1 was inoculated into 100 ml of the above medium and grown at 30 °C with shaking at 200 rpm, for 24 hours. A 5 litre shake flask was then inoculated with 50 ml of the seed culture and grown under the same conditions. The whole culture was then harvested after 48 hours growth, by

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centrifugation, and the cell paste was stored at -20 °C until use.

Preparation of Catalyst

Following thawing, Rhodococcus sp ENZA-1 cell paste (1.50 g) was suspended in phosphate buffer solution at pH 7 (0.1 M, 3.5 ml). The suspension of cells was then disrupted by sonication, to yield an essentially cell-free extract.

Preparation of (+)-lactam and (-)-amino-acid

The racemic lactam 2-azabicyclo[2.2.1]hept-5-en-3-one (218 mg, 2 mmol) was dissolved in phosphate buffer solution at pH 7 (0.1 M, 5.0 ml) and 0.5 ml of the cell-free extract was added. The resulting mixture was then inoculated at 30 °C with shaking for 14 days.

Isolation of the (+)-lactam

The reaction mixture prepared above was extracted with dichloromethane (3 x 25 ml), and the organic layers were dried with anhydrous $MgSO_4$. Following filtration, the organic layers were concentrated by rotary evaporation at 30 °C at reduced pressure, to yield a white solid (110 mg) which was fractionated by chromatography on silica (5 g) in the presence of diethyl ether as the mobile phase, to give the (+)-lactam (97 mg, 0.9 mmol; 88% e.e.).

Derivation of the (-)-amino-acid and Isolation of the (-)-ester/amide

The aqueous layer resulting from dichloromethane extraction of the reaction mixture was acidified to pH 1 with dilute HCl (1M) and then concentrated to near dryness by rotary evaporation at 35 °C and reduced pressure. The resultant oil was then refluxed with benzene (25 ml) for 1 hour in a Dean-Stark apparatus to remove water. The resulting mixture was then concentrated by rotary evaporation at 35 °C and reduced pressure, to yield a brown solid which was refluxed with dry methanol (25 ml) for 5 hours. The resulting solution was then filtered and evaporated to dryness at 35 °C and reduced pressure. Dry pyridine (10 ml) was added, and the solution was cooled in an ice-bath. At this point, acetic anhydride (5 ml) was added dropwise with stirring, and the mixture was allowed to warm to room temperature. Following a further 2 hours stirring, the solution was concentrated by evaporation at 35 °C and reduced pressure. The resulting oil was then taken up in dichloromethane (150 ml) and washed consecutively with water (30 ml), sat. $NaHCO_3$ solution (2 x 30 ml), dilute HCl (0.1 M, 2 x 30 ml) and brine (30 ml), at which point the solution was dried over anhydrous $MgSO_4$. Subsequently, the solution was concentrated by evaporation under reduced pressure at 35 °C and fractionated by chromatography on silica (10 g) in the presence of a mobile phase having the composition: $CH_2Cl_2/(CH_3)_2CO$ 80:20, to yield the (-)-ester/amide (II: X = CH_2; Y-Z = -CH=CH-; R_1 = CH_3; R_2 = Ac) (128 mg, 0.7 mmol; 81% e.e.).

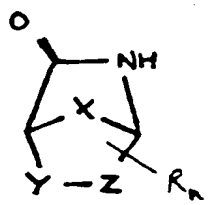
Example 2

The procedure of Example 1 was repeated, except that isolate ENZA-20 was used. In cell preparation, growth in the presence of N-acetyl-L-phenylalanine was not necessary. The products of reaction with the racemic lactam were the (-)-lactam and the (+)-amino-acid. The (-)-lactam had >98% e.e. The (+)-ester/amide was isolated.

Claims

1. An enantiomer, substantially free of other enantiomers, of a lactam of the formula

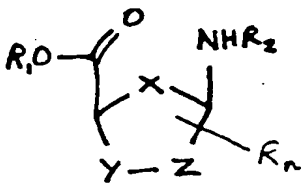
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I

wherein X is $-CH_2-$, $-(CH_2)_2-$, -Q-, $-CH_2-Q-$ or $-Q-CH_2-$ and Q is a heteroatom (including NH); either Y and Z are independently selected from $-CH_2-$ and a heteroatom (including NH), or -Y-Z- is -CH=CH-, -CH=N- or -N=CH-; and R_n is absent or represents one or more independently-selected substituents at any available position(s) on the X,Y,Z-containing ring.

2. An enantiomer, substantially free of other enantiomers, of an amino-acid or amino ester of the formula



II

wherein X, Y, Z and R_n are as defined in claim 1; R_1 is H or alkyl; and R_2 is H or a N-blocking group; provided that the enantiomer is not (+)-1R,4R-4-aminocyclopent-2-ene-1-carboxylic acid.

3. An enantiomer according to claim 2, wherein R_1 is methyl.

4. An enantiomer according to claim 2, wherein R_1 is H.

5. An enantiomer according to any of claims 2 to 4, wherein R_2 is acetyl.

6. An enantiomer according to any of claims 2 to 4, wherein R_2 is H.

7. An enantiomer according to any preceding claim, wherein R_n is absent.

8. An enantiomer according to any preceding claim, wherein X is $-CH_2-$.

9. An enantiomer according to any preceding claim, wherein -Y-Z- is -CH=CH-.

10. An enantiomer according to claim 1, which is 2-azabicyclo[2.2.1]hept-5-en-3-one.

11. An enantiomer according to claim 2, which is (-)-4-aminocyclopent-2-ene-1-carboxylic acid.

12. An enzyme having lactamase activity capable of stereospecific reaction with a mixture of enantiomers of a compound of formula I as defined in any of claims 1 and 7 to 10.

13. An enzyme according to claim 12, characterised by the ability to cause stereoselective formation of an enantiomer of 2-azabicyclo[2.2.1]hept-5-en-3-one and (-) or (+)-4-aminocyclo-pent-2-ene-1-carboxylic acid from racemic 2-azabicyclo-[2.2.1]hept-5-en-3-one.

14. A microorganism having lactamase activity as defined in claim 12 or claim 13.

15. A microorganism according to claim 14, having the characteristics of that deposited as NCIMB 40123 or NCIMB 40249, or a mutant thereof.

16. A process for preparing an enantiomer according to any of claims 1 to 11, which comprises reacting a mixture of enantiomers of the lactam of formula I with an enzyme according to claim 12 or claim 13 or a microorganism according to claim 14 or claim 15.

17. A process according to claim 16, wherein the lactam is reacted in the presence of a nucleophile, whereby the amino ester (R_1 is not H) is formed.

18. A process for preparing an enantiomer of formula II as defined in claim 2, wherein R_1 and/or R_2 are H, which comprises reacting the corresponding enantiomer wherein R_1 and R_2 are H with a nucleophile and/or an acylating agent or other agent capable of introducing a N-blocking group.

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 31 1253

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	CHEMICAL ABSTRACTS, vol. 93, no. 3, 21st July 1980, page 738, abstract no. 26739t, Columbus, Ohio, US; R.D. ALLAN et al.: "Synthesis of analogs of GABA. III. All four stereoisomers of 3-aminocyclopentanecaroxylic acid and a stereochemical correlation with amidinomycin", & AUST. J. CHEM. 1979, 32(11), 2517-21 * Abstract *	2,4,6-8	C 07 D 209/52 C 07 C 233/52 C 12 N 1/00 C 12 N 9/86 C 12 P 41/00
X	CHEMICAL ABSTRACTS, vol. 91, no. 25, 17th December 1979, page 25, abstract no. 204205j, Columbus, Ohio, US; G.A.R. JOHNSTON et al.: "Stereospecific actions of GABA analogs", & ADV. PHARMACOL. THER., PROC. INT. CONGR. PHARMACOL., 7TH 1978 (Pub. 1979), 2, 11-18 * Abstract; compounds I,III,IV *	2,4,6-8	
A	TETRAHEDRON LETTERS, vol. 28, no. 17, July 1987, pages 1887-1888, Oxford, GB; S. SICSIC et al.: "Chemoenzymatic approach to carbocyclic analogues of ribonucleosides and nicotinamide ribose" * Page 1887 *	2-5,7-9	
X	EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 24, no. 4, July-August 1989, pages 415-420, Paris, FR; M. IKBAL et al.: "Synthèse des deux énantiomères de l'analogue carbocyclique du nicotinamide ribose et évaluation de leurs propriétés biologiques" * Pages 416,419 *	2-5,7-9	
A	JOURNAL OF ORGANIC CHEMISTRY, vol. 43, no. 12, 9th June 1978, pages 2311-2320, American Chemical Society, Washington, DC, US; S. DALUGE et al.: "Synthesis of carbocyclic aminonuleosides" * Page 2311, scheme I; page 2314, right-hand column, compounds 1,2a,2b *	1-5,7-11	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 D 209/00
C 07 D 233/00
C 12 N 1/00
C 12 N 9/00
C 12 P 41/00

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	18 January 91	ENGLISH R.F.

CATEGORY OF CITED DOCUMENTS

X: particularly relevant if taken alone
Y: particularly relevant if combined with another document of the same catagory
A: technological background
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